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13. ABSTRACT (Maximum 200 Words) We have identified a complex <u>SR</u> -related <u>matrix</u> proteins of <u>160kDa</u> and <u>300kDa</u> (SRm160/300) that functions in splicing by promoting critical interactions between splicing factors bound to pre-mRNA, including snRNPs and SR family proteins (Blencowe et al., 1998; Eldridge et al., 1999). During the past year, we have investigated the functions of the SRm160/300 subunits. Surprisingly, specific depletion of SRm300 does not prevent the splicing of pre-mRNAs shown previously to require SRm160/300. Addition of recombinant SRm160 alone to SRm160/300-depleted reactions specifically activates splicing. The results indicate that SRm160 is the critical component of the SRm160/300 coactivator in the splicing of SRm160/300-dependent pre-mRNAs. This work paves the way for a more detailed investigation of interactions involving the SRm160/300 splicing coactivator that are important for constitutive and regulated pre-mRNA splicing. The carboxyl terminal domain (CTD) of the largest subunit of RNA polymerase II is important for the efficient processing of pre-mRNA- it promotes 5' end capping, splicing and polyadenylation. In order to identify factors that communicate between the transcription and the RNA processing machineries, we have used affinity chromatography to purify proteins that specifically bind to the CTD. These studies have identified a known splicing factor, PSF, and a related protein, p54 ^{nrb} , as CTD-associated proteins. We are currently determining the possible functional significance of the binding of these proteins to the CTD in relation to mechanisms underlying the coordination of transcription and splicing. These studies may contribute insights into mechanisms underlying the regulation and deregulation of alternative splicing, including altered splicing events implicated in metastatic transitions in breast and other cancers.					
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FOREWORD

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CONTENTS

PAGE

Contents	4
Introduction	5
Body	10
Discussion and Conclusions	14
Relevance to the SoW objectives	17
References	18
Publications during Year 3	24
Figure Legends	25
Figures	27

Introduction

Deregulation of alternative splicing has been linked to malignant transformation and the formation of metastases in breast cancers. For example, specific alternative spliced forms of the cell surface adhesion glycoprotein CD44 have been correlated with invasive tumor formation. Moreover, it has been demonstrated that expression of specific alternative spliced forms of CD44 mRNAs in non-metastatic cell lines result in transition to full metastatic potential. We are interested in understanding the mechanisms underlying the regulation of alternative splicing with the long term goal of identifying and targeting trans-acting splicing factors that are involved in tumor development in breast and other types of cancer.

Approximately one third of mammalian pre-mRNAs are alternatively spliced to generate protein products with functionally distinct properties (reviewed in: Smith et al., 1989; Green, 1991; Wang and Manley, 1997). Alternative splicing is a critical step in many cell differentiation and developmental pathways and must be precisely regulated. Despite the wealth of information that has emerged on the function of basal components of the spliceosome, little is known about the factors and mechanisms underlying the regulation of splice site selection.

A fundamental difference between the splicing processes of eukaryotes is the requirement for a multitude of proteins that contain domains rich in alternating arginine and serine residues (RS domains). These proteins are highly conserved in metazoans but, with few exceptions, are absent in *Saccharomyces cerevisiae*. Proteins that contain RS domains can be broadly divided into two subgroups: those of the "SR family" and SR proteins that are structurally and functionally distinct from the SR family (referred to below as "SR-related" proteins) (Fu, 1995; Manley and Tacke, 1996; Valcarcel and Green, 1996; Blencowe et

al., 1999). Both groups function at multiple stages during spliceosome formation. For example, they function early to promote the stable recognition of splice sites in constitutively spliced pre-mRNAs, and during the regulation of splice site selection in alternatively spliced pre-mRNAs. They also function at subsequent stages of spliceosome assembly, for example, by facilitating the recruitment of the U4/U6.U5 tri-snRNP particle to pre-mRNA.

SR family proteins contain one or two RNA recognition motifs (RRMs) and a phosphorylated C-terminal RS domain (Fu, 1995; Manley and Tacke, 1996; Valcarcel and Green, 1996). Members of the SR family include SRp20, ASF/SF2, SC35, 9G8, SRp30c, SRp40, SRp55, SRp75, and a more distantly related protein, p54 (Zhang and Wu, 1996). Individual members of the SR family activate splicing in cytoplasmic S100 extracts, which contain sufficient concentrations of factors required for splicing except SR proteins (Krainer et al., 1990b; Zahler et al., 1992). They promote the preferential utilization of intron-proximal splice sites in pre-mRNAs containing cis-competing splice sites (Ge and Manley, 1990; Krainer et al., 1990a; Fu et al., 1992; Cáceres et al., 1994; Wang and Manley, 1995). They also promote the recognition of adjacent splice sites in pre-mRNAs by binding to specific sequences within exons called enhancers (Lavigne et al., 1993; Sun et al., 1993; Dirksen et al., 1994; Tanaka et al., 1994; Wang and Manley, 1995).

A large number of SR-related proteins have been identified that function in splicing (Fu, 1995; Lopez, 1998; Blencowe et al., 1999). These include proteins that are homologous to yeast splicing factors; the yeast homologues however lack the RS domain. Among these are the U1-snRNP-associated 70 kDa protein and several mammalian proteins that are members of the DEXD/H-box family of RNA-dependent ATPases, which are required for conformational rearrangements in the spliceosome during splicing, and for mRNA release

from the spliceosome after splicing (de la Cruz, 1999 #748]. Many SR-related proteins however do not have known homologues or orthologs in yeast. Examples are regulators of alternative splicing that were initially defined genetically in *Drosophila*, such as suppressor of white apricot (SWAP), transformer (Tra) and transformer-2 (Tra-2). SWAP is a structurally and functionally highly conserved protein that autoregulates its expression by suppressing the splicing of its own pre-mRNA (Denhez and Lafyatis, 1994; Spikes et al., 1994). Tra and Tra-2 function as part of a cascade of alternative splicing events involved in the regulation of sex-determination in *Drosophila* (Inoue et al., 1992; Tian and Maniatis, 1993; Tian and Maniatis, 1994). Tra contains an RS domain but lacks an RRM, whereas Tra-2 contains a single RRM located between two RS domains. These proteins form a multi-subunit complex with SR family proteins on an exonic splicing enhancer (ESE) sequence within exon 4 of the *doublesex* (dsx) pre-mRNA. Formation of this complex on exon 4 promotes the utilization of an upstream, suboptimal, 3' splice site resulting in exon 4 inclusion and, ultimately, differentiation into female flies. In the absence of formation of this complex, exon 4 is excluded leading to the male differentiation pathway. Recently, two mammalian homologs of *Drosophila* Tra-2 (hTra2 α and hTra2 β) have been identified (Dauwalder et al., 1996; Beil et al., 1997). These proteins preferentially bind to mammalian ESEs containing GAA-repeats and promote ESE-dependent splicing from these sequences in mammalian cell extracts (Tacke et al., 1998).

Another level at which pre-mRNA splicing may be regulated is through interactions with RNA polymerase II. All pre-mRNAs are synthesized by RNA polymerase II (pol II). A unique structural feature of the largest subunit of pol II is that it contains a carboxyl-terminal domain (CTD) consisting of multiple tandem repeats of the heptapeptide consensus sequence: YSPTSPS (Allison et al., 1985; Corden et al., 1985). This domain is absent from pol I and pol III, which are responsible for the synthesis of non-intron

containing stable RNAs. The CTD is highly conserved in eukaryotes, although there is a larger number of heptapeptide repeats within the CTD in more complex organisms (*S. cerevisiae* has 26 or 27 whereas mammals have 52 repeats). The CTD is a substrate for several kinases, particularly on the S and T residues (reviewed by (Dahmus, 1996). Changes in the level of phosphorylation of the CTD have been correlated with different stages of the transcription cycle: pol II has a relatively low level of CTD phosphorylation (pol IIa) at pre-initiation, whereas pol II associated with transcription initiation and elongation has a hyperphosphorylated CTD (pol IIo). Although the CTD is not obligatory for the transcriptional activity of pol II, several studies have identified requirements for the CTD in enhanced transcription activity (reviewed by: (Koleske and Young, 1995; Parvin and Young, 1998). Since the CTD is a specific feature of pol II, it had been hypothesized that it could play a critical role in pre-mRNA processing (Corden, 1990). A model was proposed in which the negatively charged CTD of elongating polymerase interacts with SR proteins through their RS domains, which were proposed to have a net positive charge (Greenleaf, 1993). Support for aspects of this insightful model have been obtained recently following studies providing evidence for multiple functional roles for the CTD in pre-mRNA processing, as well as evidence for specific interactions between the CTD and pre-mRNA processing factors, including a set of novel SR-related proteins (Corden and Patturajan, 1997; Steinmetz, 1997).

In recent studies supported by the USDoD Breast Cancer Research Program, we have identified a splicing coactivator, SRm160/300 (complex of the SR-related nuclear matrix proteins of 160 kDa and 300 kDa), that is important for the processing of a subset of constitutively spliced pre-mRNAs (Blencowe et al., 1998). In last year's report results were described providing evidence that SRm160/300 is required for a purine-rich mammalian ESE to promote splicing of a pre-mRNA derived from the *Drosophila doublesex* gene (dsx pre-mRNA) (see attached manuscript by: (Eldridge et al., 1999)).

SRm160/300 likely promotes splicing of these substrates by forming multiple interactions with factors bound directly to a pre-mRNA; its association with pre-mRNA requires U1 snRNP, SR proteins bound to an ESE, and is stabilized by U2 snRNP.

During the past year we have extended our analysis of the SRm160/300 splicing coactivator. Part I of this report describes experiments aimed at determining the individual functions of the SRm160/300 subunits. SRm300 is not required for splicing of pre-mRNAs previously shown to be dependent on SRm160/300. Consistent with this finding, we demonstrate that SRm160 alone activates splicing in reactions depleted of SRm160/300. These and related results described in last year's report are described in a manuscript we have recently submitted to *RNA Journal*.

Part II of this report summarizes the results of a new project aimed at the identification and characterization of factors that specifically associate with different forms of the largest subunit of RNA polymerase II. In these studies, we have identified two proteins with links to splicing that are associated with the RNA pol II CTD. These results of these findings further support the proposal that pre-mRNA splicing and transcription are physically coupled through the CTD, and also provide a new avenue for investigating the mechanism underlying this coupling.

BODY

In last year's report, the cloning of cDNAs encoding SRm300 and a functional characterization of the SRm160/300 complex in exonic-enhancer-dependent splicing was described. In Part I of this year's report, a more detailed characterization of the SRm160/300 subunits in splicing is described. In Part II of the report, the results of a biochemical strategy to identify new RNA polymerase II CTD-associated proteins are described. A discussion of how the results of the past year address the Statement of Work (SoW) for year 3 of the CDA and a set of data figures follows the body of the report.

Part I:

SRm300 is not required for splicing of constitutive and exonic enhancer-dependent pre-mRNAs.

Previously, we demonstrated that SRm160/300 is required for the splicing of a subset of constitutively spliced pre-mRNAs, and also for activation of splicing by an exonic splicing enhancer (ESE) sequence consisting of six GAA-repeats (Eldridge et al., 1999). This ESE promotes the splicing of a pre-mRNA derived from exons 3 and 4 of the *doublesex* gene of *Drosophila* (*dsx*(GAA)₆ pre-mRNA). However, these studies did not address whether one or both of the SRm160/300 subunits are important for splicing of these substrates. To investigate this, nuclear extract specifically immunodepleted of SRm300 with rAb-SRm300 was tested for splicing activity (Figure 1). Unlike rAb-SRm160, which efficiently co-immunodepletes SRm300 with SRm160 (Blencowe et al., 1988; see Figure 3 in this previous study), immunodepletion with rAb-SRm300 efficiently depletes SRm300 without resulting in a significant reduction in the level of SRm160 (Figure 1A, compare lanes 2 and 3). Immunoblot analysis of the pellet fractions from the rAb-SRm300

immunodepletion revealed that only a minor level of SRm160 was co-immunoprecipitated with rAb-SRm300 (data not shown). This indicates that, unlike rAb-SRm160, rAb-SRm300 may disrupt the SRm160/300 complex during immunodepletion. However, it is also possible that a significant fraction of SRm160 is in excess of SRm300 in nuclear extract. In contrast to depletion of SRm160/300 with rAb-SRm160 (Figure 1B,C lanes 3), depletion of SRm300 alone with rAb-SRm300 did not inactivate splicing of the PIP85A pre-mRNA (Figure 1B lane 5), or the dsx(GAA)₆ pre-mRNA (Figure 1C, lane 5). This indicates that SRm160, but not SRm300, is the more important component of the SRm160/300 splicing coactivator for the splicing of these substrates.

SRm160 activates splicing in SRm160/300-depleted reactions

The above observations suggested that SRm160 alone might complement an extract depleted of both SRm160 and SRm300. To test if this is the case, a recombinant SRm160 (rSRm160) protein containing six N-terminal histidine residues was expressed in insect cells from a baculovirus vector and purified to homogeneity (refer to Materials and Methods). The final fraction from the purification contained a single polypeptide migrating at ~180 kDa (Figure 2A, lane 1). This corresponds to the mobility of a fraction of SRm160 in HeLa nuclear extract that is highly phosphorylated, indicating that rSRm160 is also highly phosphorylated. Immunoblotting with antibodies to SRm160 confirmed the identity of the recombinant protein as SRm160 (data not shown).

Addition of increasing amounts of rSRm160 to splicing reactions containing nuclear extract immunodepleted of SRm160/300 activated splicing of PIP85A pre-mRNA (Figure 2B, compare lane 1 with lanes 2-4). The highest level of splicing activity obtained upon addition of rSRm160 corresponded to approximately 30% of the level of splicing activity in a control reaction containing a mock-depleted nuclear extract (compare lane 4 with lane

1). Addition of higher amounts of rSRm160 resulted in a decrease in splicing efficiency (data not shown). As was observed previously for biochemically purified SRm160/300, rSRm160 did not activate splicing when added to a cytoplasmic S100 extract, confirming that it is distinct in activity from SR family proteins, which activate splicing in these extracts (data not shown).

Addition of levels of rSRm160 to SRm160/300-depleted reactions that activated splicing of the PIP85A pre-mRNA did not activate splicing of the dsx(GAA)₆ pre-mRNA. Although it cannot be excluded that an additional component besides SRm160 is missing from the depleted extract that is specifically required for ESE function, this seemed unlikely since the immunodepletion procedure did not alter the level of any SR family or hTra 2 protein detected by the anti-SR protein antibody mAb104 (Blencowe et al. 1998, refer to Figure 3 in this previous study; data not shown). Subsequently, it was found that levels of rSRm160 that activated PIP85A splicing in the SRm160/300 depleted reaction were inhibitory to splicing of the dsx(GAA)₆ pre-mRNA in a mock-depleted nuclear extract. These findings suggest that splicing of the dsx(GAA)₆ pre-mRNA may be difficult to reconstitute in SRm160/300-depleted reactions because this substrate is particularly sensitive to altered ratios of SRm160 to other splicing factors. Nevertheless, the results demonstrate that specific depletion of SRm160, but not SRm300, prevents both constitutive and ESE-dependent splicing, and that rSRm160 alone can activate splicing in the absence SRm300.

Part II

The pre-mRNA splicing factor PSF/p54nrb associates with the CTD of RNA polymerase II.

HeLa nuclear extract was passed over different bacterially expressed GST fusion proteins containing either a full-length (52 repeats) or a partial (15 N-terminal repeats) carboxyl-terminal domain (CTD) of RNA polymerase II. These CTD ligands were either utilized in chromatography non-phosphorylated or pre-phosphorylated by incubation in nuclear extract to afford a comparison of proteins that bind differentially to phosphorylated vs. non-phosphorylated CTD polypeptides (see introduction). Two prominent proteins of ~55 and 110 kDa were detected in the eluates from the non-phosphorylated and phosphorylated long CTD columns (Figure 3, lanes 6 and 7). Proteins of same sizes were also detected but at much lower levels in the eluates from the short CTD columns (lanes 4 and 5). The binding of these proteins did not appear to be influenced by the phosphorylation status of the CTD ligands. MALDI-TOF mass spectroscopy was used to determine the masses of peptides obtained by trypsin digestion of the 55 and 110kDa proteins. Searches of the databases for corresponding masses of peptides from conceptually translated sequences identified matches with p54^{nrb} and PSF, respectively. PSF (the polypyrimidine tract-binding protein (PTB) associated splicing factor) is an essential factor which, as its named suggests, interacts with PTB. PTB has been reported to function in the regulation of alternative splicing. p54^{nrb} (nuclear RNA-binding protein, p54) is 71% identical to PSF within a 320 amino acid region that contains two RRM. Curiously, both of these proteins have also been identified in a 1:1:1 complex with DNA topoisomerase I, which has been reported to have SR protein kinase activity. The identification of PSF/p54nrb as a CTD associated component is intriguing in light of the importance of the CTD in RNA processing. The CTD-PSF/p54nrb interaction has independently been identified by Dr. C.J. Ingles and his colleagues. We are currently preparing a joint manuscript with Dr. Ingles that will be included in next year's report.

Discussion and Conclusions

Part I

In research performed during Year 3 of the CDA it was demonstrated that, in contrast to depletion of SRm160/300, specific depletion of SRm300 does not prevent splicing of the PIP85A pre-mRNA, nor splicing of an exonic splicing enhancer (ESE)-dependent pre-mRNA derived from the *Drosophila doublesex* gene (*dsx*(GAA)₆; see introduction). When added alone to splicing reactions, recombinant SRm160 specifically activates splicing. The results provide evidence that SRm160 is the more important component of the SRm160/300 complex in splicing reactions in vitro.

SRm300 is remarkable for its high content of SR dipeptide repeats and other repetitive sequence features. The presence of extensive SR repeat-rich domains in SRm300 indicates that it is capable of interacting simultaneously with many factors, including SRm160 and other SR proteins. Such a function could be important for the organization of SR proteins and other splicing factors within the cell nucleus (see below). The two very long polyserine tracts in SRm300 are reminiscent of serine-rich domains that have been identified in other nuclear proteins including Nopp140, SCAF10 and RNPS1. Nopp140 is a nucleolar phosphoprotein that appears to shuttle along "tracks" between the nucleolus and cytoplasm (Meier and Blobel, 1992). SCAF10 is an SR-related cyclophilin concentrated in speckles that is associated with the carboxyl terminal domain of RNA polymerase II large subunit (Bourquin et al., 1997). RNPS1 (RNA binding Protein with Serine-rich domain) is a recently identified splicing factor that contains an RRM but lacks an RS domain (Mayeda et al., 1999). Like SRm160/300, RNPS1 stimulates splicing in reactions containing limiting concentrations of SR family proteins. However, unlike

SRm160/300, which is required for the splicing of specific pre-mRNA substrates, RNPS1 appears to function as a more general activator of splicing.

The results in the present study extend our earlier observations indicating that SRm160/300 functions as a coactivator in the splicing of specific constitutively spliced pre-mRNAs and also an ESE-dependent pre-mRNA. In the previous studies, it was demonstrated that the association of SRm160/300 with a pre-mRNA requires U1 snRNP, SR proteins bound to an ESE, and is stabilized by U2 snRNP (Blencowe et al., 1998; Eldridge et al., 1999). Moreover, in the absence of pre-mRNA, it was found that SRm160/300 interacts weakly but specifically with U2 snRNP and with a subset of SR proteins including the ESE-binding protein hTra2 β (Eldridge et al., 1999). The results of the present study suggest that, in the absence of SRm300, SRm160 most likely mediates critical interactions between these components bound to pre-mRNA.

The results indicate that SRm300 is dispensable for the splicing of specific pre-mRNAs that require SRm160. However, this does not exclude the possibility that it is required for the splicing of other substrates, and/or that it has functions in splicing not detected *in vitro*. In particular, the observation that SRm300 is stably associated with SRm160 and with splicing complexes through both steps of the splicing reaction on different pre-mRNA substrates suggests that it participates in splicing. Several kinases have been identified recently including members of the SRPK and Clk/Sty families, which phosphorylate RS domains and are known to influence protein-protein interactions between SR proteins and splicing activity (Misteli, 1999). These kinases could also influence critical interactions in splicing involving SRm160/300, which are both phosphoproteins and contain phosphorylated RS domains (Blencowe et al., 1995). Such a mechanism could operate at a more indirect level in the case of SRm300. For example, changes in the level of phosphorylation of SRm300, which is tightly bound to the nuclear

matrix in speckle structures, could influence the nuclear distribution of SRm160 and/or other associated SR proteins, and thereby modulate splicing activity by regulating the availability of these proteins at sites of nearby transcription. These and other possibilities will require more detailed investigations of the functions of the SRm160/300 splicing coactivator subunits. The results during the past year advance our understanding of mechanisms underlying the splicing of specific pre-mRNAs and, ultimately, will facilitate investigations into the mechanisms by which splicing is deregulated during metastatic transitions in breast cancer.

Part II

The identification of PSF and p54^{nrb} as proteins that associate with the CTD of RNA polymerase II provides further evidence for a physical connection between RNA polymerase II and pre-mRNA processing. Previously, we reported that hyperphosphorylated RNA polymerase II is associated with snRNPs, SR proteins, and also assembled splicing complexes (Mortillaro et al., 1996). The present findings extend these results and provide clues as to the nature of factors that communicate between transcription and splicing. Future work will be aimed at determining the possible functional significance of the PSF/p54^{nrb}-CTD interaction.

Relevance to the Statement of Work (SoW) objectives.

Refer to the revised SoW from August 25 1998. Experiments described in the reports for Years 1 and 2, and the present report, center on defining the function of the SRm160/300 complex and the identification of sequences that recruit it to exonic RNA. These experiments address and extend the objectives outlined in the SoW Tasks 1, 3, 5 and 6. Specific experiments such as the cross-linking strategy outlined in Task 3 will not be performed since we have already defined by alternative methods pre-mRNA sequences and factors by which SRm160/300 associates with pre-mRNA. The experiments described in Part II of this report directly address the objectives of Task 2 in the revised SoW, which were to analyze interactions between the transcription and splicing machinery. We are currently analyzing the influence of overexpression of wild type and mutant derivatives of SRm160 on the nuclear organization of splicing factors and splicing in vivo. These studies will address item 6 of the SoW and will be summarized in next year's report.

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Figure Legends

Figure 1. Immunodepletion of SRm300 does not block splicing

A. Immunodepletion of SRm300. Nuclear extract immunodepleted with rAb-SRm300 (lane 3) or mock depleted with pre-immune serum (lane 2) was separated alongside a sample of untreated nuclear extract (lane 1) on a 7% SDS polyacrylamide gel and immunoblotted with mAb-B4A11 (specific for SRm300) and rAb-SRm160.

B. Immunodepletion of SRm300 does not block splicing of PIP85A pre-mRNA. Splicing reactions incubated for 1hr with PIP85A pre-mRNA contained a regular (untreated) nuclear extract (lane 1), nuclear extracts depleted of SRm160/300 (lane 3) or SRm300 (lane 5), and nuclear extracts mock-depleted with pre-immune sera (lanes 2 and 4) from rabbits immunized with GST-SRm160 and GST-SRm300 fusion proteins, respectively. RNA recovered from the splicing reactions was separated on a 15% denaturing polyacrylamide gel. The splicing reaction intermediates and products are indicated.

C. Immunodepletion of SRm300 does not prevent splicing of an ESE-dependent pre-mRNA.

Splicing reactions were incubated for 1hr with a pre-mRNA derived from exons 3 and 4 of the *Drosophila doublesex* gene, which contains a mammalian ESE consisting of six GAA repeats (dsx(GAA)₆ pre-mRNA). The reactions contained the same nuclear extracts as described in 3B. RNA recovered from the splicing reactions was separated on a 7% denaturing polyacrylamide gel.

Figure 2. Reconstitution of SRm160/300-depleted splicing reactions with recombinant SRm160.

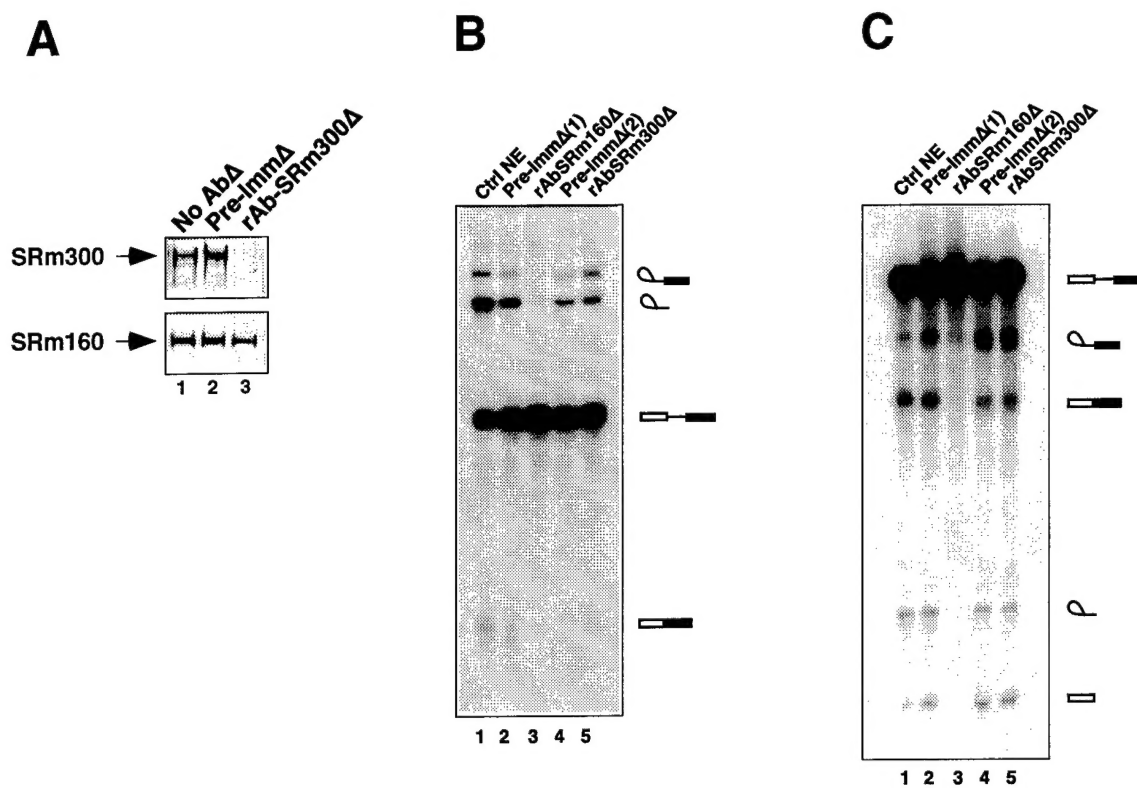
A. Recombinant SRm160 containing six N-terminal histidine residues (rSRm160) was expressed in insect cells using a baculovirus vector, purified to homogeneity (see Materials and Methods), and was separated on a 12% SDS polyacrylamide gel and stained with Coomassie (lane 1). Lane 2 shows a sample of molecular weight standards (sizes are indicated in kDa).

B. rSRm160 promotes splicing of PIP85A pre-mRNA in SRm160/300-depleted reactions. Splicing reactions incubated for 1hr contained nuclear extract mock-depleted with pre-immune serum (lane 1) or SRm160/300-depleted nuclear extract supplemented with 0, 1.5, or 3.0 μ l of rSRm160 (~100 μ g/ μ l) (lanes 2-4, respectively). 3.0 and 1.5 μ l of protein buffer was added to the reactions in lanes 2 and 3, respectively, to control for buffer effects. RNA recovered from the splicing reactions was separated on a 15% denaturing polyacrylamide gel.

Figure 3. Isolation of CTD-associated proteins by CTD-affinity chromatography

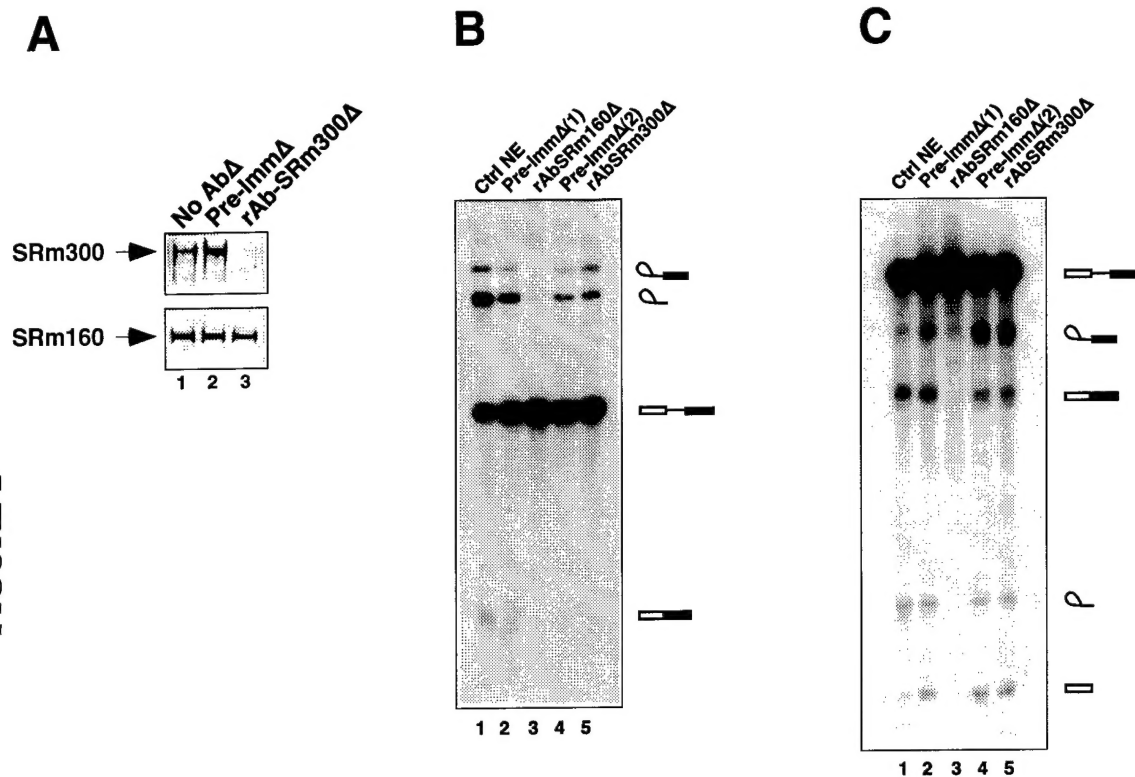
Silver stained SDS polyacrylamide gel showing eluates from control and CTD resins. A DNase and RNase-treated fraction from HeLa cell nuclear extract (load) was chromatographed on resins coupled to the GST-CTD and control polypeptides indicated. The Load sample (lane 1) represents 15% of the total load, whereas the eluates represent 30 % of the total sample collected.

FIGURE 1



Blencowe et al.

FIGURE 2



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FIGURE 3

